A SIMPLIFIED BACTERIOLOGICAL TECHNIQUE FOR DETECTING CHOLERA CARRIERS.*

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The routine bacteriological examination of immigrants from cholera-infected ports, as practiced at the Quarantine Station at New York, has been as follows:

1. Inoculation of feces into Dunham's peptone solution.
2. Subinoculation at the end of six hours of one loop of the surface growth into a second Dunham's peptone tube.
3. Examination of a smear taken from the surface growth of the second Dunham's peptone tube, after it has been incubated six to nine hours at 37°C.†

This procedure has in general given very satisfactory results but it necessitates considerable microscopical work. On several occasions at New York it has been necessary to examine over two thousand immigrants a day, and even a trained force of bacteriologists required a whole day to complete the examination of so many smears, so that an untrained staff for such an examination would certainly require several days. The substitution of a macroscopic test for the microscopic examination, would greatly lessen the work.

To make such a substitution possible, I began a series of experiments on the cultural characteristics of the cholera organism. The cholera-red reaction does not give satisfactory results in mixed cultures. I demonstrated that the cholera vibrio ferments many of the sugars, especially saccharose, which is fermented rapidly without gas formation. The problem, then, was how to utilize this phenomenon. As the cholera organism is very susceptible to acids, the acid produced by fermentation of the sugar is detrimental to its growth, and as the cholera vibrio grows readily in alkaline media, the natural solution of the problem was to use an alkaline medium, for therein the acid elaborated during the growth of the vibrio would be neutralized by the alkalinity. Furthermore the alkali would inhibit many of the other intestinal organisms, some of which ferment saccharose. As Dunham's peptone solution has proven such a favorable

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† For more detailed description of this method see the article by Dr. Creel on page 899 of this issue.

medium for the cholera vibrio the new medium was made by simply adding saccharose and sodium carbonate to the Dunham's peptone solution until the medium was alkaline, phenolphthalein being added as an indicator.

The medium consists of,

Water	1000 cc.
Peptone	10 gms.
Salt	5 gms.
Sodium Carbonate	1 gm.
Saccharose	5 gms.
Phenolphthalein Solution	5 cc.

The salt and peptone are dissolved in the water over a free flame. The medium is then titrated with sodium carbonate using phenolphthalein as an indicator and is next corrected to a neutral reaction. One gram of anhydrous sodium carbonate is dissolved in the solution which is then boiled over a free flame, after which it is filtered through a double layer of filter paper. Five grams of saccharose and five cubic centimeters of a fifty per cent. saturated alcoholic solution of phenolphthalein are next added. The medium should then be of a moderately deep red color similar to the color of 0.1 per cent. fuchsin solution. One familiar with the medium may simplify the procedure by omitting the titration and correction of the reaction. In many cities tap water may be used in making up the solution, and the carbonate may be added with the salt and peptone. If after adding the phenolphthalein the medium is not deep red enough, a 10 per cent. sodium carbonate solution should be added till the medium is brought to the desired shade. If the medium is to be used the day it is made up, one sterilization in the Arnold sterilizer for twenty minutes is sufficient; if it is to be kept for some time, the medium must be sterilized in the Arnold for fifteen minutes on three consecutive days.

In this medium, if a moderate number of cholera vibrios are introduced together with other organisms, the culture becomes decolorized after five to eight hours incubation. The cholera vibrios ferment the saccharose, the acid produced unites with the sodium carbonate and the medium becomes neutral, hence the red color of the phenolphthalein disappears. As a minimal number of cholera organisms introduced with a maximal number of other organisms do not readily decolorize the culture, the cholera vibrios should be enriched by preliminary incubation in Dunham's peptone solution.

The technique in using this medium is as follows:

1. A small quantity of feces is inoculated into Dunham's peptone solution and incubated six hours at 37°C.

2. The tube containing the incubated culture is carefully tilted and one loop of the surface growth therein is inoculated into saccharose peptone.

3. The saccharose peptone is incubated at 37°C. A control is made by inoculating tubes of saccharose peptone with one loop of a fresh culture of cholera in Dunham's peptone solution and one loop of a fecal culture in Dunham's peptone solution and placing one of these tubes in each incubator. At the end of five hours all the tubes are examined, and any that have become decolorized, or nearly so, are removed for additional examination. They are re-examined at the end of every hour until the tubes have been incubated eight hours. By that time the controls should be completely decolorized. Those tubes that are not decolorized within eight hours may be discarded safely, provided the controls are decolorized completely. The only important factor is the time within which the tubes are decolorized. After fifteen hours incubation at 37°C. over 90 per cent. of the tubes become decolorized, but those tubes containing vibrios are decolorized within five to eight hours. Decolorization of the upper layer of the peptone is strongly indicative of the presence of vibrios.

Examination of the decolorized tubes. These should be examined as soon as they become decolorized and before the medium becomes acid. A smear is made from the surface growth and is stained with dilute carbolfuchsin. It is then examined and if any vibrios are seen, a third subinoculation from the surface growth is made into Dunham's peptone solution; the specimen is also plated immediately on plain agar or on one of the special cholera media. If the period for the examination is limited, time can be saved by inoculating a duplicate set of Dunham's peptone tubes at the same time that the saccharose peptone tubes are inoculated. Those tubes that become decolorized are then examined in the duplicate Dunham's peptone solution and subcultures and hanging drop agglutinations may be made therefrom.

The chief advantage of the procedure here outlined is that it greatly reduces the number of smears to be examined. By examining smears from the subcultures one trained bacteriologist can examine only two to three hundred specimens a day, but with the aid of the saccharose peptone medium, he can examine two to three thousand specimens a day even if he has only untrained assistants to make the inoculations and subinoculations. The chief use of this medium is therefore in the examination of a large number of suspects and in emergencies where no trained force is available. The procedure is of little use where only a few specimens are to be examined.

The only disadvantages which I have encountered in this procedure are (1) that the smears made from the surface growth are not as satisfactory as are those made from Dunham's peptone solution, and (2) that the specimen is poorer for the examination of motility and of hanging-

drop agglutinations. The disadvantages may be overcome by making duplicate subcultures in Dunham's peptone solution. The difficulties probably arise from the presence of a fermentable sugar in the medium, for oxygen is obtainable from the saccharose, and consequently the aerobic condition of the upper strata of the fluid is not so essential to the vibrios as it is in the Dunham's peptone solution, and therefore they show less tendency to collect at the surface.

At the Quarantine Station at New York I tested this method on 3730 suspects. In the saccharose peptone medium 106 cultures were decolorized within eight hours. Four non-cholera vibrios were isolated. The method was controled by examining smears from all the specimens. Vibrios were found in the decolorized tubes only. I was handicapped by the absence of cholera cases and carriers, none having been present at New York Quarantine for several months. However, each group of specimens was controlled by an artificial cholera inoculated stool.

Experimental data:

1. In order to show how favorable the medium is for the cholera vibrio, equal quantities of a twelve hour cholera culture were inoculated into Dunham's peptone solution and into saccharose peptone. At the end of six hours one cubic centimeter of each was plated and the colonies counted. The saccharose peptone showed more colonies per cubic centimeter than did the Dunham's peptone solution, but the surface growth as shown by smears was more abundant in the latter.

2. Accuracy of the method:—Varying dilutions of cholera cultures were made by the addition of fecal cultures in Dunham's peptone solution. In no case where the mixture was previously enriched by first incubating in Dunham's peptone solution did the organisms fail to decolorize the medium within eight hours.

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